

**Supplementary information for: Designed α -sheet peptides
suppress amyloid formation in *Staphylococcus aureus*
biofilms**

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Table S1. Peptide designs used in this study

All designs are single-turn α - or β -sheet hairpins, with the exception of *RC* (random coil). The peptides are N- and C-terminally acetylated and amidated, respectively, except for β , which has a free N-terminus. Lower case and underlined letters denote D-amino acids, turn residues are shown in red, and disulfide-bonded cysteines in AP407 are shown in bold.

Controls	<i>RC</i>	Ac-KLK <u>p</u> LLTSENTL-NH ₂
	β	SWTW <u>E</u> <u>p</u> <u>N</u> <u>K</u> <u>W</u> TWK-NH ₂
α-sheet Designs	<i>AP90</i>	Ac-RGE <u>m</u> <u>N</u> <u>l</u> <u>S</u> <u>w</u> <u>M</u> <u>N</u> <u>E</u> <u>Y</u> <u>S</u> <u>G</u> <u>w</u> <u>t</u> <u>M</u> <u>n</u> <u>L</u> <u>k</u> <u>M</u> <u>G</u> <u>R</u> -NH ₂
	<i>AP401</i>	Ac-r <u>G</u> <u>e</u> <u>M</u> <u>n</u> <u>L</u> <u>s</u> <u>W</u> <u>m</u> <u>n</u> <u>e</u> <u>y</u> <u>s</u> <u>G</u> <u>w</u> <u>T</u> <u>m</u> <u>N</u> <u>l</u> <u>K</u> <u>m</u> <u>G</u> <u>r</u> -NH ₂
	<i>AP407</i>	Ac-RGE <u>m</u> <u>N</u> <u>l</u> <u>C</u> <u>w</u> <u>M</u> <u>N</u> <u>E</u> <u>Y</u> <u>S</u> <u>G</u> <u>w</u> <u>c</u> <u>M</u> <u>n</u> <u>L</u> <u>k</u> <u>M</u> <u>G</u> <u>R</u> -NH ₂
	<i>AP193</i>	Ac-RGE <u>m</u> <u>N</u> <u>y</u> <u>F</u> <u>w</u> <u>M</u> <u>N</u> <u>E</u> <u>Y</u> <u>Y</u> <u>G</u> <u>w</u> <u>t</u> <u>M</u> <u>n</u> <u>C</u> <u>k</u> <u>M</u> <u>G</u> <u>R</u> -NH ₂

Table S2. Bacterial strains used in this study

<i>Strain</i>	<i>Description</i>
<i>S. aureus</i> SH1000 (WT)	Laboratory strain, <i>rsbU</i> ⁺
<i>S. aureus</i> MN8	Clinically relevant strain; urogenital tract
<i>S. aureus</i> MN8 + <i>mCherry</i>	Transcriptional fusion of RFP to the P3 promoter of strain MN8

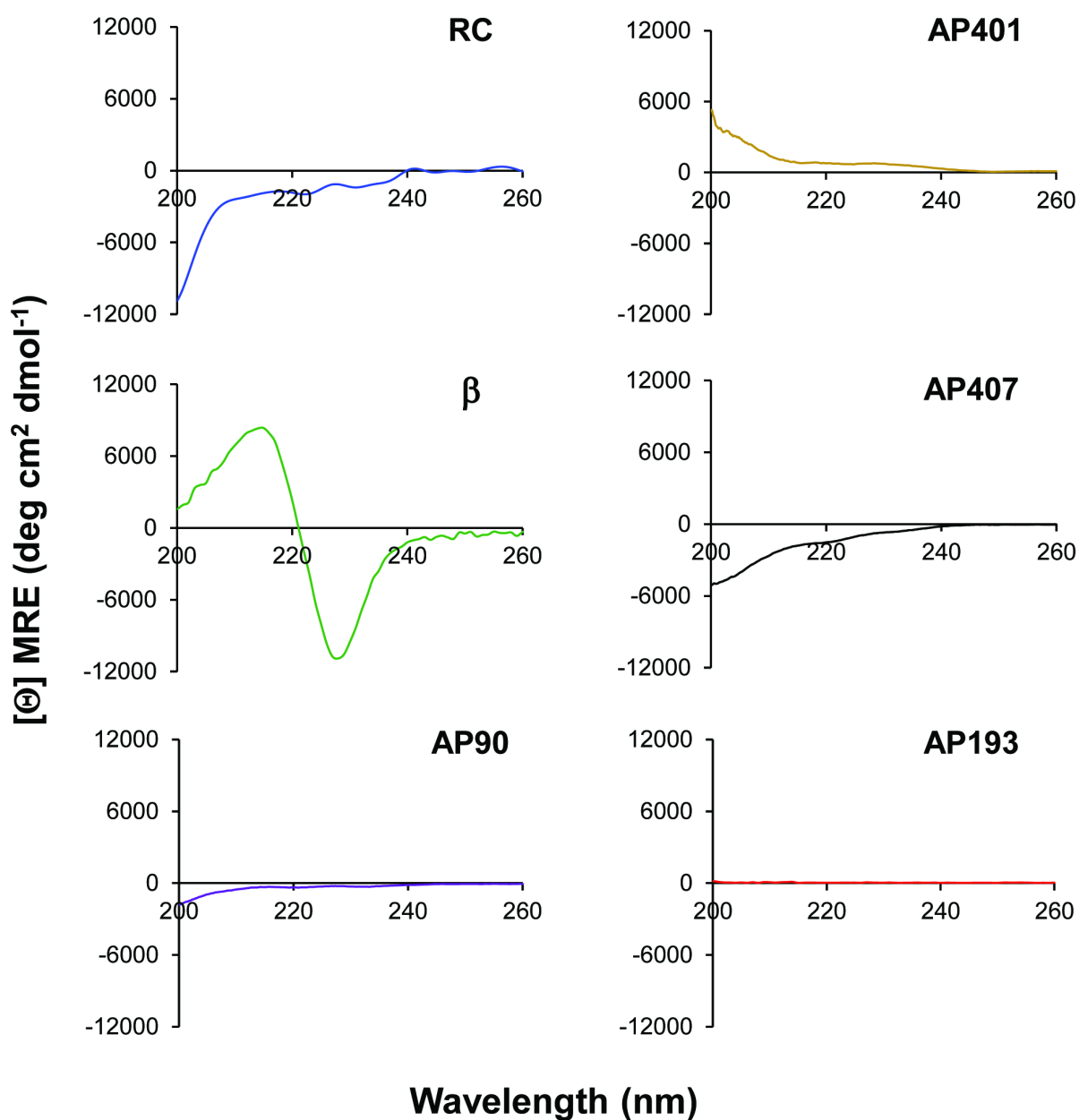


Fig S1. CD spectra of RC, β , AP90, AP401, AP407, and AP193. Peptides were dissolved to a concentration of 100 μ M (except for RC, which was 30 μ M) in phosphate buffer (except for β and AP193, which were in acetate buffer) and spectra were acquired at room temperature. $[\Theta]$ MRE = mean residue ellipticity. Note that the β -sheet spectrum of β is obscured by the Cotton effect manifested by the interaction of W-W residues across the sheet, and further structural information for this peptide has been presented elsewhere (Cochran et al., Tryptophan zippers: stable, monomeric β -hairpins. Proc. Natl. Acad. Sci. U.S.A. 2001, 98, 5578–5583).

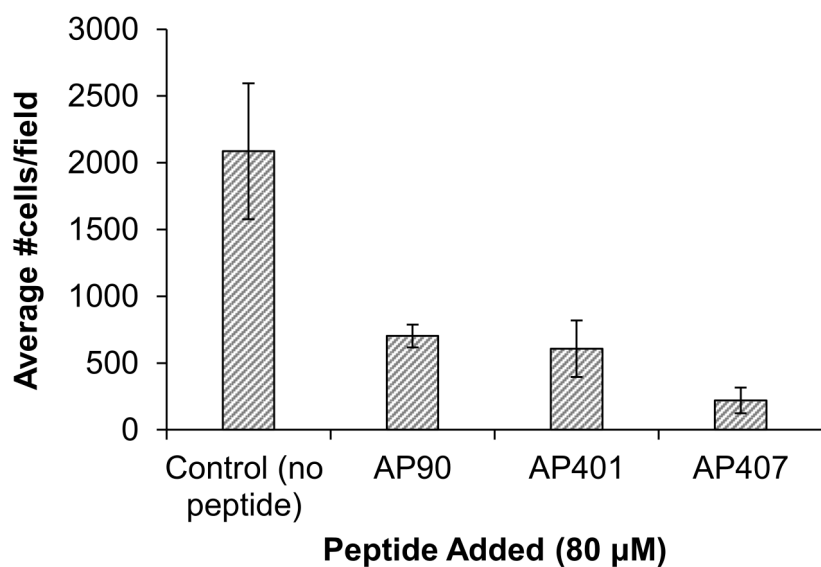


Fig. S2. Designed peptides reduce the number of cells attached to glass slides after washing. The average number of cells per field was determined for each image collected and averaged over triplicate images. Error bars represent the standard deviation of the mean. This figure goes with the images in Figure 2.

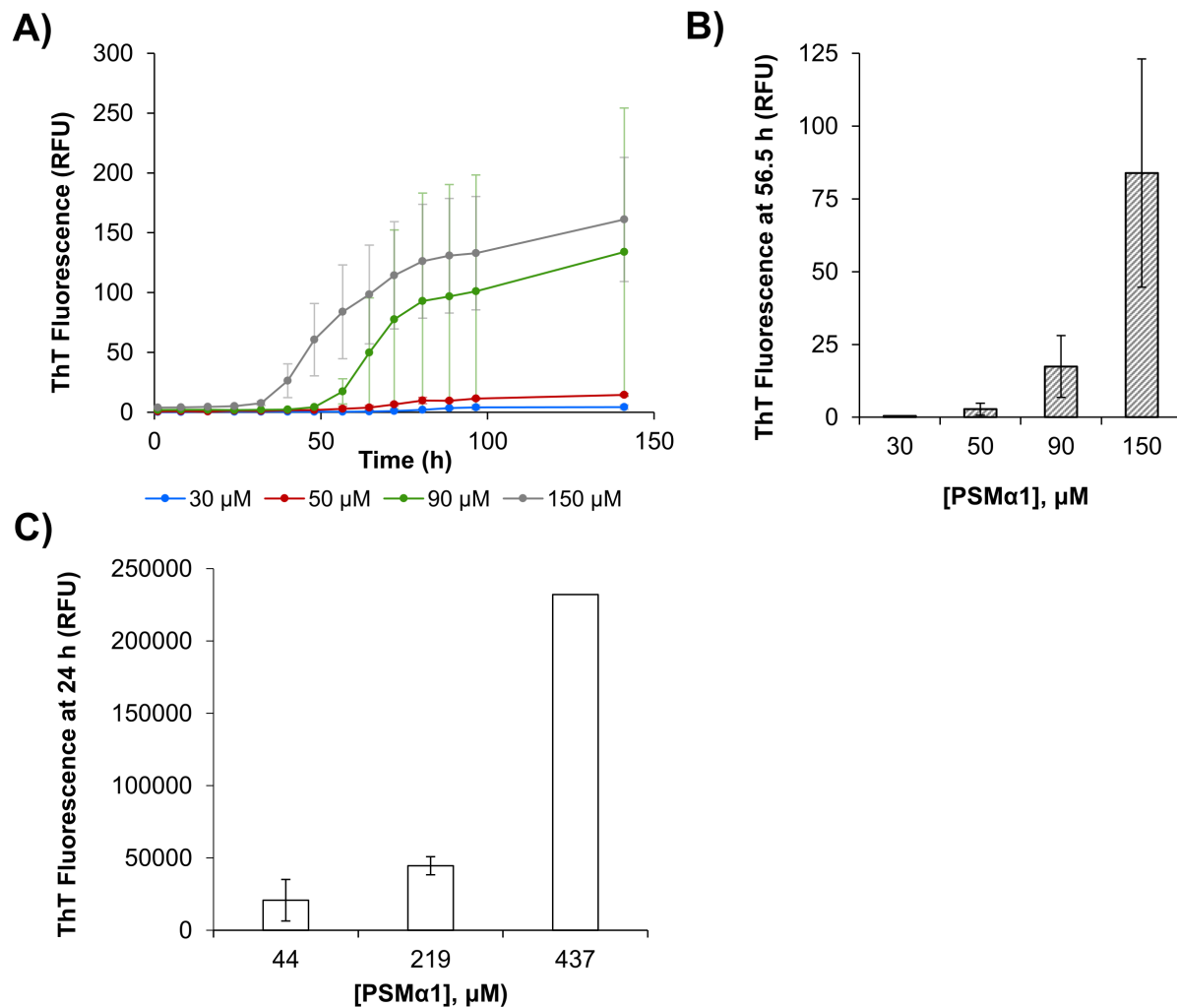


Fig. S3. The rate and extent of amyloid formation by synthetic PSM α 1 depends on peptide concentration. **(A)** Synthetic PSM α 1 peptide (0.34% DMSO in water, pH 5.5) was allowed to aggregate at four different concentrations (30, 50, 90, and 150 μ M) and amyloid formation was monitored by ThT fluorescence. Higher concentrations of PSM α 1 resulted in faster rates amyloid formation. **(B)** ThT fluorescence measurements from panel A shown for 56.5 h, which indicates there was greater extent of amyloid formation for higher concentrations of PSM α 1. **(C)** In the case where PSM α 1 was allowed to aggregate for AFM experiments, a similar correspondence between ThT fluorescence values and PSM α 1 concentration was observed. Experiments were performed in triplicate, and error bars indicate the standard deviation from the mean.

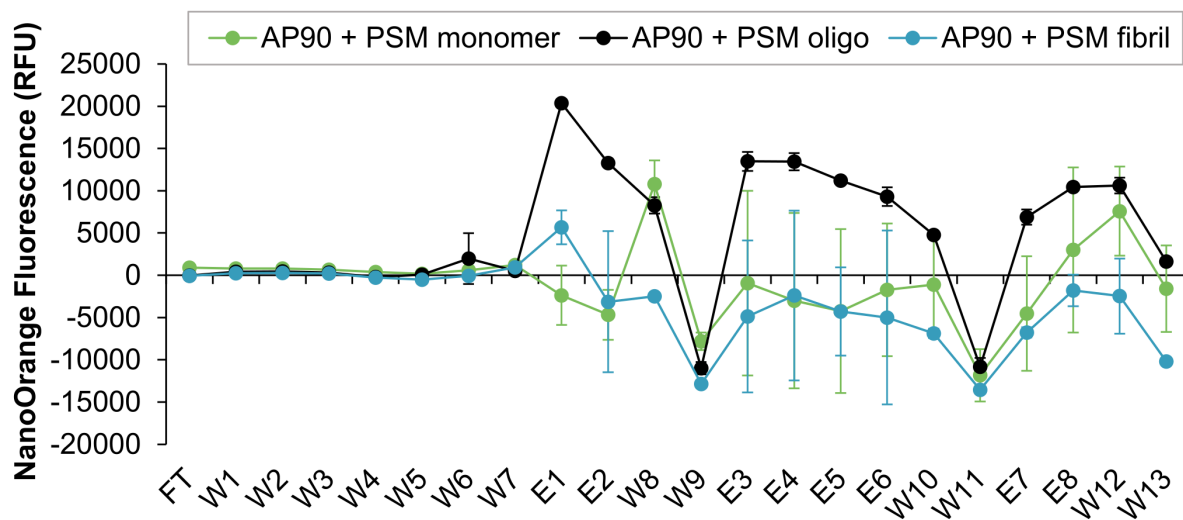


Fig. S4. The designed peptide AP90 was dissolved to a concentration of 250 μ M in PBS + 50 mM NaCNBH₃ and immobilized on Pierce Amino Link agarose beads in a Pierce spin column (Thermo Fisher Scientific) overnight at 4°C. Residual active sites were blocked with 1 M Tris HCl + 50 mM NaCNBH₃ for 4 h at 25°C. Meanwhile, PSM α 1 samples were prepared as in the “PSM fibrillization assay” (Figure 6A) and incubated at 37°C. Solutions of either fresh (0 h), pre-incubated (allowed to aggregate for 48 h corresponding to α -sheet oligomer species), or fibrillar (165 h) PSM α 1 were added to the prepared spin columns and allowed to bind to the peptide-functionalized resin beads for 2 h at 25°C. The solution was then collected by centrifugation (flow-through, FT). The beads were re-suspended in 300 μ L PBS, vortexed to obtain a uniform slurry, and then the solution was collected by centrifugation (washes, W1-W7). GndHCl was then added to the columns at three different concentrations (E1-E2, 2 M GndHCl; E3-E6, 4 M GndHCl; E7-E8, 6 M GndHCl) to elute bound PSM α 1. The GndHCl elution steps were alternated with PBS washes (W8-W13). All eluents were then analyzed with the NanoOrange® Protein Quantitation Kit and fluorescence was used as an indicator of PSM α 1 content in each eluent. Fluorescence measurements were normalized by the corresponding protein-free (no PSM α 1 added) eluents from a blank (active sites blocked but no AP90) spin column. Error bars represent the standard error of the mean over six samples.

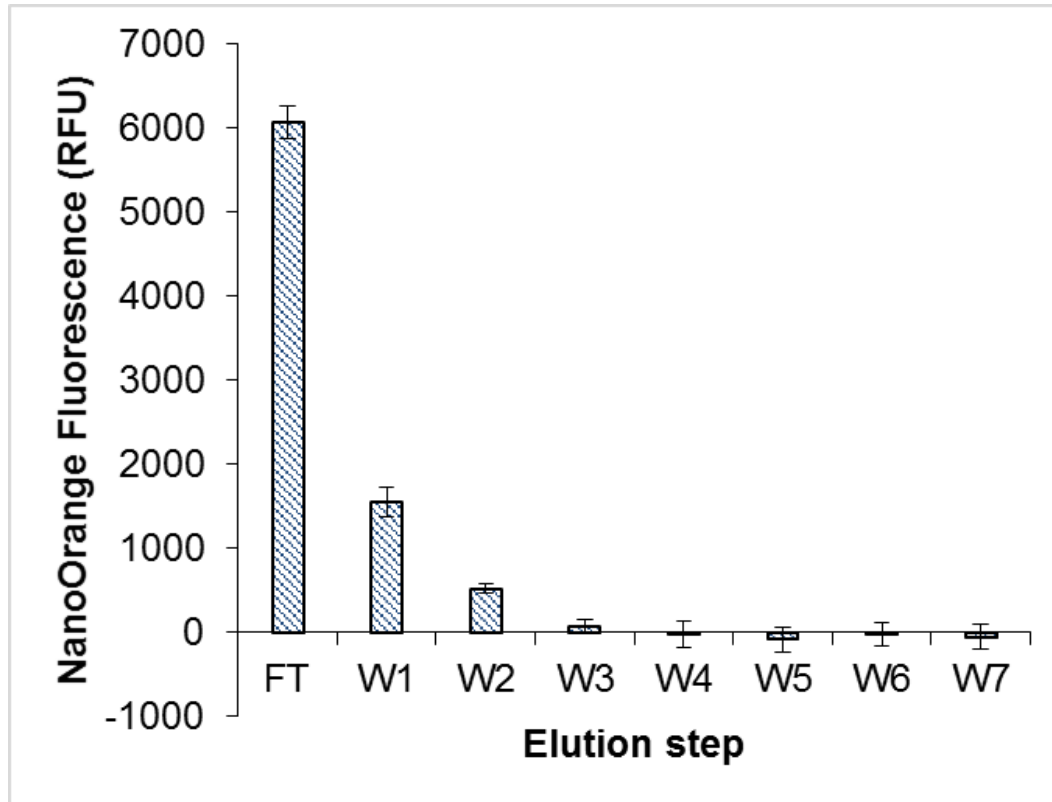


Fig. S5. Wash steps in the column-binding assay utilizing bound AP193 are sufficient to remove all detectable protein in the eluent. By the seventh wash, protein concentrations fall below the fluorescent-detectable level of 10 ng/mL. The data shown here are for fresh PSM α 1 (0 h) samples applied to the column, but they are representative of all column-binding assays. Error bars represent the standard error of the mean over six samples.